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# Study on europium doped hydroxyapatite nanoparticles by Fourier transform infrared spectroscopy and their antimicrobial properties

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## Abstract

In this work, Fourier Transform Infrared spectroscopy (FT-IR) analysis were conducted on europium doped hydroxyapatite,  $\text{Ca}_{10-x}\text{Eu}_x(\text{PO}_4)_6(\text{OH})_2$  nanocrystalline powders (Eu:HAp) with  $0 \leq x_{\text{Eu}} \leq 0.2$ . Antimicrobial studies were also performed for the first time on Eu:HAp. The antimicrobial properties of Eu:HAp nanoparticles with  $0 \leq x_{\text{Eu}} \leq 0.2$  on Gram-negative (*E coli* ATCC 25922, *Pseudomonas aeruginosa* 1397) and Gram-positive (*Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212) bacteria systems and a species of fungus (*Candida albicans* ATCC 10231) were reported. Our study demonstrates that the antimicrobial activity of Eu:HAp nanoparticles is dependent on the europium concentration.

**Keywords:** nanocrystalline, hydroxyapatite, europium, antimicrobial activity.

## 1. Introduction

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is the main inorganic component of human bones and teeth, showing a very good biocompatibility, bioactivity and osteoconductivity due to its nontoxic, and noninflammatory properties [1-9]. Therefore, it has been widely used in many fields, like bio-medical applications, as a bioactive coating material for metallic implants, clinical bone augmentation, dental implantology, tumors treatment and cell activation, or it could be also used as a carrier in drug delivery systems [10]. Moreover, trivalent rare-earth-

ion-doped hydroxyapatite can also be used as biological fluorescent probes due to their excellent luminescent properties.

In the last years, luminescence imaging, especially in the region of near-infrared spectrum showed a great interest for biomedical applications, focusing on the development and characterization of rare-earth based inorganic luminescent nanoparticles [11-14]. The most important applications could be found on pharmaceutical industry or biological and medical diagnostics [15-17].

When doped with rare-earth ions, hydroxyapatite is being considered a fluorescent probe, the luminescence intensity depending on the concentration of rare-earth's doping ions, the degree of crystallinity and the crystal structure of the host material [18-20]. Over the past decade, several methods for obtaining luminescent inorganic nanoparticles have been developed, including coprecipitation and sol-gel synthesis which allow to adjust particles morphology, size, structure and composition in order to adapt their physical and chemical properties [14, 21-23].

Fluorescent labeling is an indispensable technique which is widely used for performing nondestructive observations both in vivo and in vitro by replacing the calcium ions in hydroxyapatite crystal lattice using rare earth luminescent ions [24-26]. The hexagonal hydroxyapatite allows the substitutions of many rare earth ions without any changes in the crystal structure [27-30]. Nowadays, the rare earths ions doped hydroxyapatite nanoparticles are being studied very intently as cell labeling materials, as a result of their strong luminescence under visible light spectrum. [31-36]

Compared to other rare earth elements, trivalent Europium  $\text{Eu}^{3+}$  ions have a simple electronic energy level scheme and hypersensitive transitions. The  $\text{Eu}^{3+}$  doped calcium apatites represent a good biological probe candidate due to their low toxicity and stable luminescence over time and it has been proven that a small amount of europium in the bioactivity behaviour has no harmful effects [37-41]. The ionic radius similarity between  $\text{Ca}^{2+}$  and  $\text{Eu}^{3+}$  in the apatite lattice makes them a good host for  $\text{Eu}^{3+}$  doping [4].

Recently, great attention has been paid to europium doped hydroxyapatite (Eu:HAp) potential use as biological probe [30]. Previously, the europium doped hydroxyapatite was studied applying PL [32-33]. On the other hand, IR spectroscopy is a powerful method in ionic investigation and has been used extensively in phosphate minerals research [42-44].

Concerning the phase composition of the as-prepared Eu:HAp various studies have been reported [31-33, 45]. In our recent studies no other additional phases were observed [45] for the synthesized Eu:HAp samples and the diffraction patterns were identical to that

obtained for stoichiometric apatite. In this paper, the obtained Eu:HAp samples were systematically characterized by Fourier transform infrared (FTIR) spectroscopy. The aim of this work is to contribute to the study of the influence of europium in the structure of hydroxyapatite. On the other hand, cell viability from various doses of the Eu:HAp samples at two different time points were compared with corresponding culture media values. After 24 h and 48h exposure to all types of Eu:HAp at 10 µg /ml concentration, the morphology of the cells was preserved. Moreover, we report in this work for the first time the bacterial studies on Eu:HAp. Furthermore, in this article, original studies on antimicrobial activities of Eu:HAp against Gram-positive (*Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212), Gram-negative (*E coli* ATCC 25922, *Pseudomonas aeruginosa* 1397) and fungal strains (*Candida albicans* ATCC 10231) are presented.

## **2. Materials and methods**

### **2.1. Samples**

All the reagents for synthesis, including ammonium dihydrogen phosphate  $[(\text{NH}_4)_2\text{HPO}_4]$ , calcium nitrate  $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$ , and europium nitrate  $[\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}]$  (Alpha Aesare) were used as purchased, without purification. Europium doped hydroxyapatite (Eu:HAp,  $\text{Ca}_{10-x}\text{Eu}_x(\text{PO}_4)_6(\text{OH})_2$ ) nanoparticles were performed by setting  $x_{\text{Eu}} = 0.01$ ,  $x_{\text{Eu}} = 0.02$ ,  $x_{\text{Eu}} = 0.05$ ,  $x_{\text{Eu}} = 0.1$ ,  $x_{\text{Eu}} = 0.2$  and  $[\text{Ca}+\text{Eu}]/\text{P}$  as 1.67 in accord with [45].

### **2.2. Fourier transforms infrared (FTIR) spectroscopy.**

The functional groups present in the prepared nanoparticles and thin films were identified by FTIR using a Perkin Elmer, Spectrum BX spectrometer. In order to obtain the nanoparticles spectra, 1% of the nanopowder was mixed and ground with 99% KBr. Tablets of 10 mm diameter were prepared by pressing the powder mixture with a pressure of not more than 10 psi. The spectrum was recorded in the range of 400 to 4000  $\text{cm}^{-1}$  with 4  $\text{cm}^{-1}$  resolution. The first FTIR spectra were obtained after 256 scans at room temperature ( $25 \pm 0.5^\circ\text{C}$ ). The second derivative IR spectra were acquired after 5-point smoothing of the original IR spectra. For selected spectral ranges, the peak fitting analyses were performed using procedures of J. Kolmas et al. [46]: (i) baseline correction, (ii) second derivative

calculation and self deconvolution assessment in order to determine the number and positions of the bands and (iii) curve fittings with fixed peak positions using Lorentzian lines. In the previous studies Matsuhiro et al. [47] and Gómez-Ordóñez et al. [48] showed that second-derivatives of FTIR spectra are generally used as an aid for wave number determination of weak absorption bands onto improve resolution of overlapped bands in the original spectra. To that end, in our studies derivation including Savitzky-Golay algorithm with nine smoothing points was performed.

### 2.3. Antimicrobial studies.

The microbial strains identification was confirmed by aid of VITEK II automatic system. VITEK cards for identification and susceptibility testing were inoculated and incubated according to the manufacturer's recommendations. Microbial suspensions of  $1.5 \times 10^8$  CFU/mL corresponding to 0.5 McFarland density obtained from 15-18 h bacterial cultures developed on solid media were used in our experiments. The tested substances were solubilised in DMSO and the starting stock solution was of 5000  $\mu\text{g/mL}$  concentration. The qualitative screening was performed by an adapted disk diffusion method [49-53].

## 3. Results and discussions

### 3.1. IR Absorbance Spectra

The FTIR spectra of Eu:HAp were recorded in the typical, absorption mode from KBr pellets. IR absorbance spectra of EuHAp samples with various europium concentrations ( $0 \leq x_{\text{Eu}} \leq 0.2$ ) are shown in the  $4000 \text{ cm}^{-1}$  to  $400 \text{ cm}^{-1}$  range in Figure 1. In our previous studies [45] we have shown that for all the samples the presence of strong  $[\text{OH}]^-$  vibration peak ( $632 \text{ cm}^{-1}$ ) could be noticed. The broad bands in the regions  $1600\text{--}1700 \text{ cm}^{-1}$  and  $3200\text{--}3600 \text{ cm}^{-1}$  correspond to H–O–H bands of lattice water. Fowler B.O. in his infrared studies on apatites [54] showed that the band at  $3570 \text{ cm}^{-1}$  is characterized by  $[\text{OH}]^-$  stretching mode and the band at  $632 \text{ cm}^{-1}$  characterized by  $[\text{OH}]^-$  arises from stretching librational mode. The bands at around  $1090 \text{ cm}^{-1}$  and about  $1040 \text{ cm}^{-1}$  can be attributed to the  $\nu_3 [\text{PO}_4]^{3-}$  while the band at  $962$  arises from  $\nu_1 [\text{PO}_4]^{3-}$ . The  $602 \text{ cm}^{-1}$  and  $564 \text{ cm}^{-1}$  bands appear from  $\nu_4 [\text{PO}_4]^{3-}$ . Markovik et al. [55], presented that the sharpness of bands, especially sharpness of the  $632$

$\text{cm}^{-1}$ ,  $602\text{ cm}^{-1}$  and  $564\text{ cm}^{-1}$  bands indicate a well-crystallized HAp. The band at  $475\text{ cm}^{-1}$  can be attributed to the  $\nu_2$   $[\text{PO}_4]^{3-}$  [56]. In the FT-IR spectrum of Eu:HAp with  $x_{\text{Eu}} \geq 0.1$  (Figure 1) the bands corresponding to the  $\nu_3$  vibration of C–O were observed at  $1410\text{ cm}^{-1}$ , characteristic of the carbonate group [57-58].

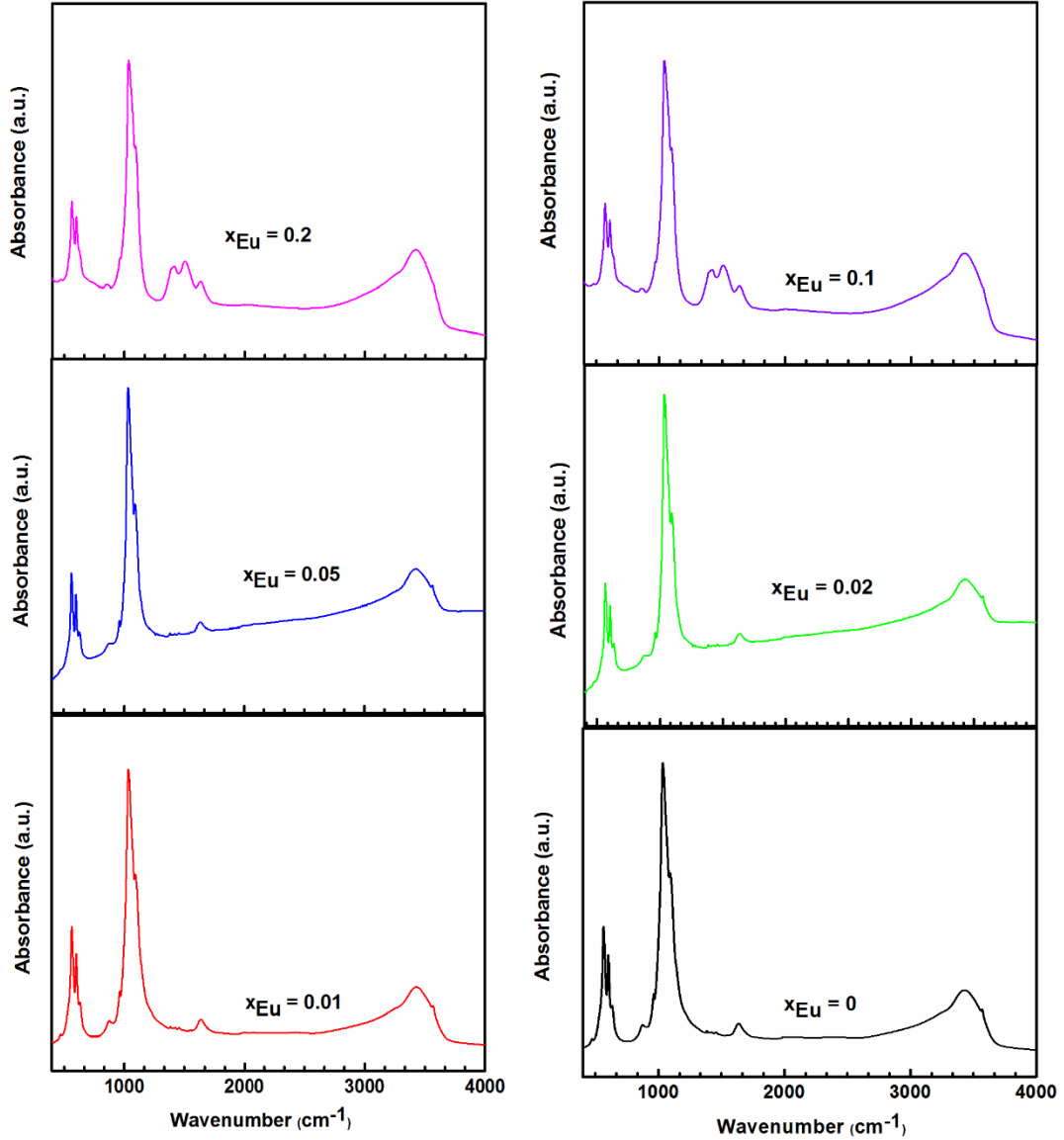


Figure 1: FT-IR spectra of europium doped hydroxyapatite (Eu:HAp) with  $x_{\text{Eu}} = 0$ ,  $x_{\text{Eu}} = 0.02$ ,  $x_{\text{Eu}} = 0.2$ .

The intensity of the band located at  $1410\text{ cm}^{-1}$  in the spectrum of Eu:HAp samples with  $x_{\text{Eu}} \geq 0.1$  is attributed to components of the  $\nu_3$  mode of a trace amount of  $[\text{CO}_3]^{2-}$ . The  $\nu_2$  vibrations between  $850$  and  $890\text{ cm}^{-1}$ , characteristic of the carbonate group were not

detected because  $\nu_2$   $[\text{CO}_3]^{2-}$  band at  $872\text{ cm}^{-1}$  is hidden by  $[\text{HPO}_4]^{2-}$  band at  $875\text{ cm}^{-1}$ . Similar comportment was observed by Markovik et al. [55] in his studies on preparation and comprehensive characterization of calcium hydroxyapatite. Markovik et al. and Holcomb D.W. et al. [59] showed that the  $[\text{CO}_3]^{2-}$  band at  $1410\text{ cm}^{-1}$  derives from  $[\text{CO}_3]^{2-}$  (designated by “B-type” carbonate that replace  $\text{PO}_4^{3-}$  ions in the hydroxyapatite lattice). The band at  $1510\text{ cm}^{-1}$  was also detected in the FT-IR spectrum of Eu:HAp with  $x_{\text{Eu}} \geq 0.1$ . The band at  $1510\text{ cm}^{-1}$  derives from  $[\text{CO}_3]^{2-}$  (designated by “A-type” carbonate) that replaces  $\text{OH}^-$  ions in the hydroxyapatite lattice [55, 60]. In all the spectra of Eu:HAp samples, the band at  $875\text{ cm}^{-1}$  was detected. This band is supposed to arise due to  $[\text{HPO}_4]^{2-}$  ions from several reasons [55].

In Figure 1 we observed that the contribution of the area that corresponds to the phosphate bands decreases when the europium concentration in the samples increases. The bands at  $475$  and  $962\text{ cm}^{-1}$  progressively disappear with the increase of europium concentration. When the  $x_{\text{Eu}} = 0.2$  the bands at  $475$  and  $962\text{ cm}^{-1}$  are almost absent. We can also observe in the Eu:HAp spectra a broadening of peak vibration with the decrease of the europium concentration. This behaviour was observed by Owada et al. [61] in sintered Y-doped hydroxyapatite.

### 3.2. IR Second Derivative Spectra

In order to complete structural information on the analyzed Eu:HAp samples with  $0 \leq x_{\text{Eu}} \leq 0.2$  we agreed to perform derivative analysis and peak fitting of selected  $\nu_4$ ,  $\nu_3$ ,  $\nu_2$  and  $\nu_1$  phosphate bands. The second derivative of the spectrum of Eu:HAp samples ( $x_{\text{Eu}} = 0$ ,  $x_{\text{Eu}} = 0.02$ ,  $x_{\text{Eu}} = 0.2$ ) in the  $\nu_4$ ,  $\nu_3$  and  $\nu_1$   $[\text{PO}_4]^{3-}$  bands are shown in Figure 2. Only the results obtained for pure HAp ( $x_{\text{Eu}} = 0$ ) and Eu:HAp ( $x_{\text{Eu}} = 0.02$ ,  $x_{\text{Eu}} = 0.2$ ) samples are shown, which clearly assigned the strong changes that occur in the FT-IR spectra of HAp in the presence of Eu.  $\nu_1$   $[\text{PO}_4]^{3-}$  band was observed at around  $962\text{ cm}^{-1}$  in the second derivative spectra. On the other hand, in the second derivative spectra was identified a  $\nu_2$   $[\text{PO}_4]^{3-}$  band at  $475\text{ cm}^{-1}$ . In concordance with precedent studies, the bands assigned in the second derivative spectra of Eu:HAp ( $0 \leq x_{\text{Eu}} \leq 0.2$ ) can be attributed to molecular vibrations of the phosphate  $[\text{PO}_4]^{3-}$  in an apatitic stoichiometric environment of hydroxyapatite [62].

To evaluate the subtle spectral changes occurring as a consequence of the europium doped hydroxyapatite, the spectra in the spectral regions of  $450\text{--}700\text{ cm}^{-1}$  ( $\nu_2$  and  $\nu_4$   $[\text{PO}_4]^{3-}$

domain) and 900–1200  $\text{cm}^{-1}$  ( $\nu_1$  and  $\nu_3$   $[\text{PO}_4]^{3-}$  domains) were analyzed by means of second derivative (Figure 2) and curve fitting analysis (Figure 3).

In Figure 2 we also observe the second derivative band at 633  $\text{cm}^{-1}$  that derives from the  $\text{OH}^-$  librational mode. IR wavenumber position ( $\text{cm}^{-1}$ ) of the  $\nu_4$ ,  $\nu_3$ ,  $\nu_2$  and  $\nu_1$   $[\text{PO}_4]^{3-}$  bands of Eu:HAp spectrum from second derivative is presented in Table 1. Ten bands were detected for phosphate bands of hexagonal Eu:HAp samples.

Table 1. IR wavenumber position ( $\text{cm}^{-1}$ ) of  $\nu_4$ ,  $\nu_3$ ,  $\nu_2$  and  $\nu_1$   $[\text{PO}_4]^{3-}$  bands of Eu:HAp spectrum from second derivative.

Assignments	Position ( $\text{cm}^{-1}$ )	References
$\nu_1$ $[\text{PO}_4]^{3-}$	962	62
$\nu_2$ $[\text{PO}_4]^{3-}$	475	62
$\nu_3$ $[\text{PO}_4]^{3-}$	1033; 1045; 1073; 1090	62
$\nu_4$ $[\text{PO}_4]^{3-}$	569; 575; 588; 602	62
Present in newly precipitated apatite.	1111	62-63
$[\text{HPO}_4]^{2-}$ - containing apatites	1144	63
$[\text{OH}]^-$ group ( librational mode)	633	63-64

Figure 3 shows the FT-IR spectrum of hydroxyapatite ( $x_{\text{Eu}} = 0$ ), the phosphate  $\nu_4$ ,  $\nu_3$ ,  $\nu_2$  and  $\nu_1$  regions with experimental and calculated contours overlaid along with the individual subbands (blue) as determined by a curve fitting analysis. Five components were needed for a satisfactory fit in the spectral region of 450–700  $\text{cm}^{-1}$  ( $\nu_2$  and  $\nu_4$   $[\text{PO}_4]^{3-}$  domain) and eight in the spectral region of 900–1200  $\text{cm}^{-1}$  ( $\nu_1$  and  $\nu_3$   $[\text{PO}_4]^{3-}$  domains). FT-IR spectrum of Eu:HAp with  $x_{\text{Eu}} = 0.02$  is also presented and five components were needed for a satisfactory fit in the spectral region of 450–700  $\text{cm}^{-1}$  ( $\nu_2$  and  $\nu_4$   $[\text{PO}_4]^{3-}$  domain) and six in the spectral region of 900–1200  $\text{cm}^{-1}$  ( $\nu_1$  and  $\nu_3$   $[\text{PO}_4]^{3-}$  domains). For Eu:HAp samples with  $x_{\text{Eu}} = 0.2$  four components were needed for a satisfactory fit in the spectral region of 450–700  $\text{cm}^{-1}$  ( $\nu_2$  and  $\nu_4$   $[\text{PO}_4]^{3-}$  domain) and five important components in the spectral region of 900–1200  $\text{cm}^{-1}$  ( $\nu_1$  and  $\nu_3$   $[\text{PO}_4]^{3-}$  domains).



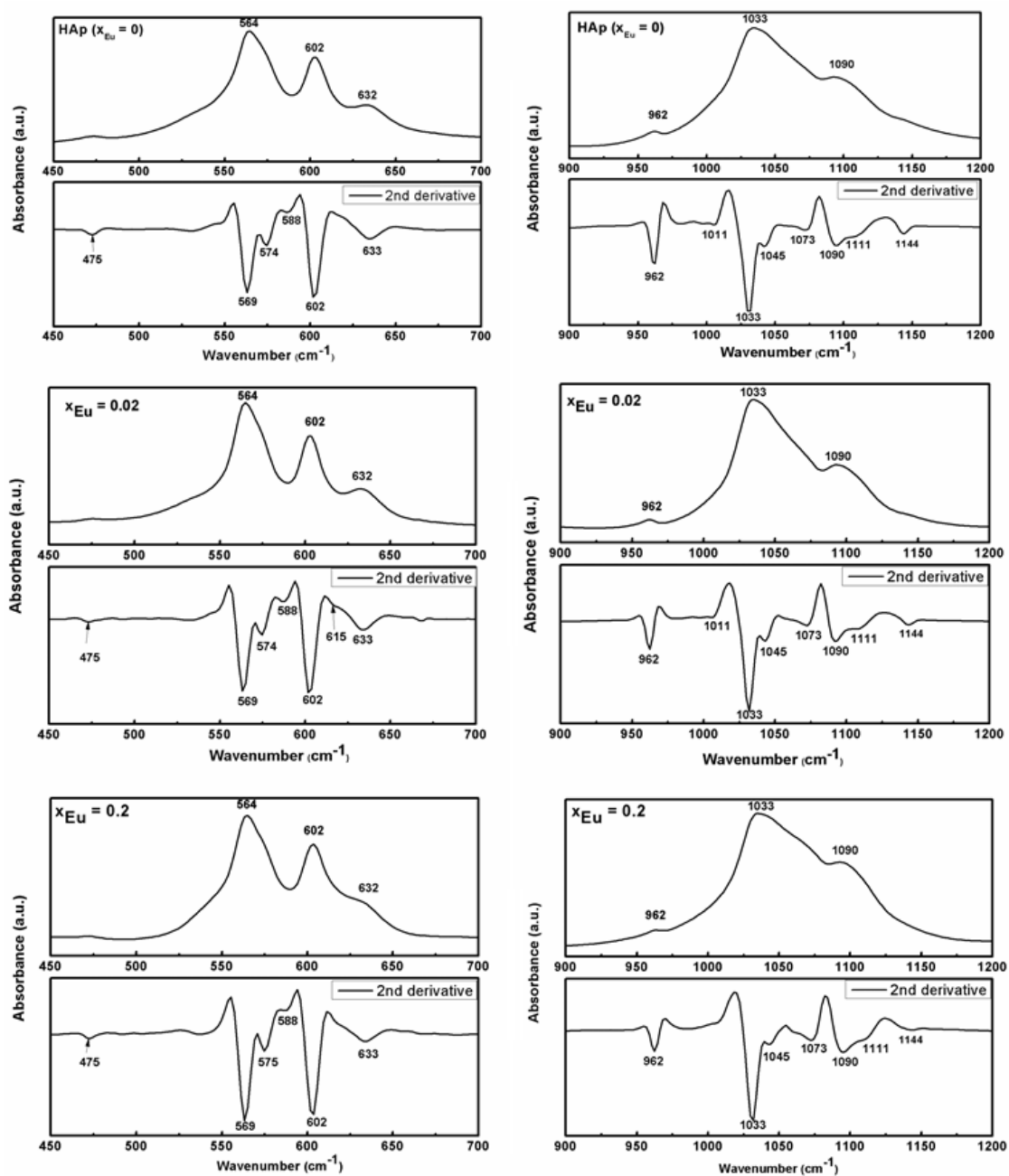


Figure 2: Second derivative of Eu:HAp ( $x_{Eu} = 0, x_{Eu} = 0.02, x_{Eu} = 0.2$ ) spectrum of the  $\nu_4, \nu_3, \nu_2$  and  $\nu_1$  domains.

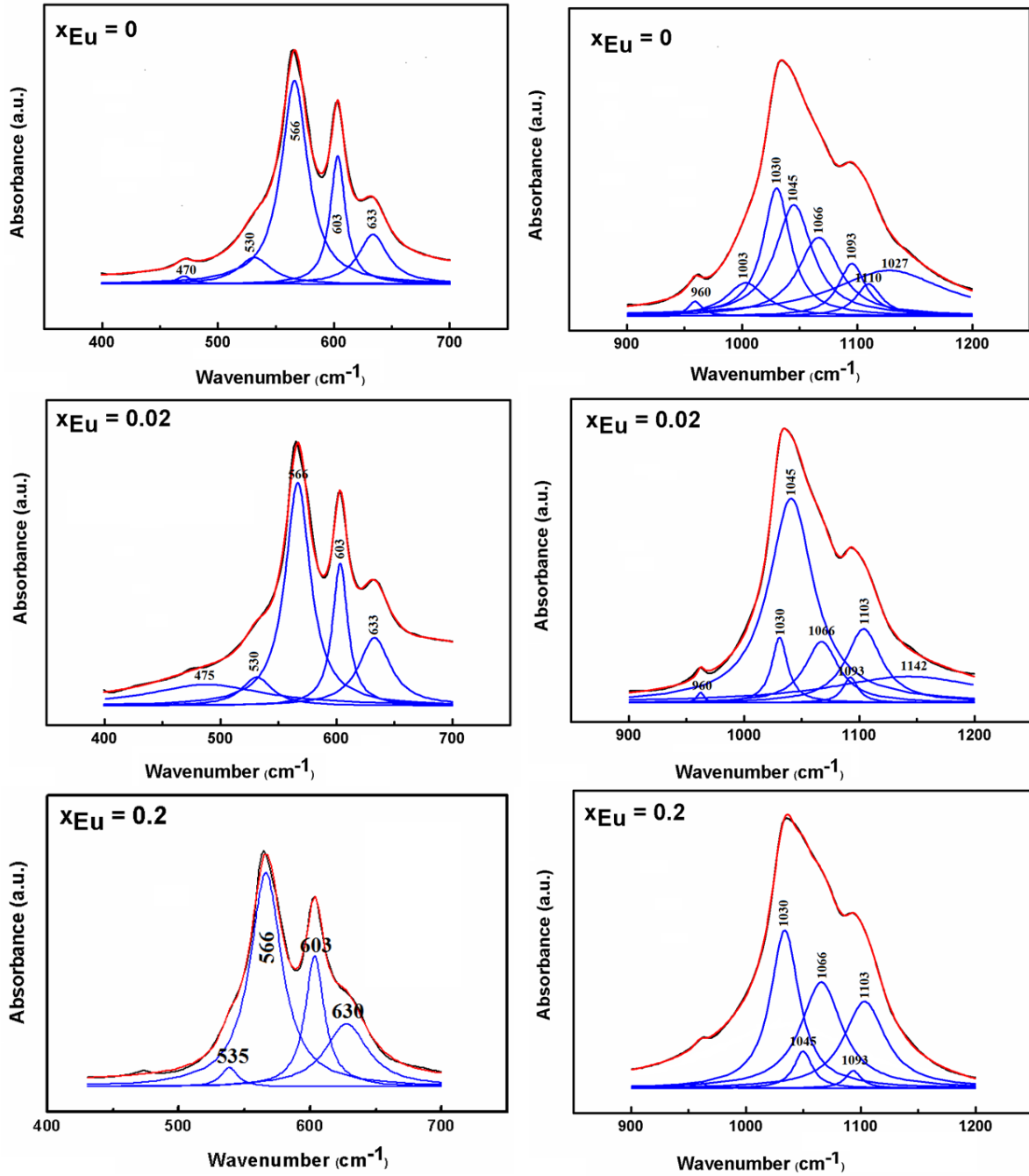


Figure 3: FT-IR deconvoluted spectra of the  $\nu_4$ ,  $\nu_3$ ,  $\nu_2$  and  $\nu_1$  domain for Eu:HAp ( $x_{Eu} = 0$ ,  $x_{Eu} = 0.02$ ,  $x_{Eu} = 0.2$ ).

The individual component for phosphate  $\nu_2$  region (featured near 475 cm<sup>-1</sup>) decreases for the samples with  $x_{Eu} = 0.02$  and disappears when  $x_{Eu}$  increases to 0.2. The high frequency shoulder is more clearly defined for pure HAp than in the case of the Eu:HAp with  $x_{Eu} = 0.02$

and  $x_{\text{Eu}} = 0.2$  in the  $\nu_1$  and  $\nu_3$  phosphate regions. The band requires at least eight components for an adequate fit to the spectrum, compared with only six in the case of Eu:HAp with  $x_{\text{Eu}} = 0.02$  or only five in the Eu:HAp with  $x_{\text{Eu}} = 0.2$ . In this series of materials it was not realistic to attempt to identify particular components characteristic the particle size. However, a correlation was found between the percentage area of the  $\nu_1$ , band (near  $960\text{ cm}^{-1}$ ) and the crystal size. The individual component for the phosphate  $\nu_1$  region (band near  $960\text{ cm}^{-1}$ ) disappears when  $x_{\text{Eu}}$  increases to 0.2. On the other hand, the individual component for phosphate  $\nu_3$  region (band near  $1040\text{ cm}^{-1}$ ) decreases for the samples with  $x_{\text{Eu}} = 0.02$ . However, in the Figure 2 and 3, we observe the disappearance of the band at  $1144\text{ cm}^{-1}$ , which is associated with  $[\text{HPO}_4]^{2-}$  ions [65]. We note that the main molecular species that gave rise to the Eu:HAp ( $x_{\text{Eu}} = 0$ ,  $x_{\text{Eu}} = 0.02$ ,  $x_{\text{Eu}} = 0.2$ ) absorbance in the  $900\text{-}1200\text{ cm}^{-1}$  region was assigned to the phosphate ion,  $[\text{PO}_4]^{3-}$ .

### 3.3. Antimicrobial studies

A study on enhancement of osteoblast proliferation on europium doped hydroxyapatite has rarely been reported. Anselme K. [66] in his study concerning osteoblast adhesion on biomaterials and Garcia AJ, et al. [67] in the study of bio-adhesive surfaces to promote osteoblast differentiation and bone formation showed that often synthetic materials do not support osteoblast adhesion and this may result in poor cell differentiation and limited bone formation. Keselowsky BG et al. [68] in the study on surface chemistry and Mc Farland CD et al.[69] in his study on protein adsorption and cell attachment to patterned surfaces demonstrated that the effect of surface properties on cellular response depends on differences in species, concentration, and biological activity of adsorbed proteins, which may be obtained from different sources, i.e., biological fluids and cell-mediated synthesis and deposition.

The antimicrobial activity of Eu:HAp ( $0 \leq x_{\text{Eu}} \leq 0.2$ ) nanoparticles was tested using the most common bacterial pathogens and fungus: *E coli* ATCC 25922 (Gram-negative), *Pseudomonas aeruginosa* 1397 (Gram-negative), *Staphylococcus aureus* 0364 (Gram-positive), *Enterococcus faecalis* ATCC 29212 (Gram-positive) and *Candida albicans* ATCC 10231 (fungus).

The antimicrobial studies on Eu:HAp ( $0 \leq x_{\text{Eu}} \leq 0.02$ ) nanoparticles indicated that antimicrobial activity is present. The results of antimicrobial activity of Eu:HAp ( $0.05 \leq x_{\text{Eu}} \leq 0.2$ ) nanoparticles are shown in Figures 4-6. For the as-prepared Eu:HAp samples an

antibacterial activity was not observed on *E. coli* ATCC 25922 (Figures 4-6). The Eu:HAp nanoparticles with  $x_{Eu} = 0.05$  showed a good antibacterial activity on *Enterococcus faecalis* ATCC 29212) (Figure 6) for all the concentrations studied (from 0.031 mg/ml to 1 mg/ml). For the samples of Eu:HAp with  $x_{Eu} = 0.1$  and  $x_{Eu} = 0.2$  (Figures 5-6) we have observed that the inhibition of *Enterococcus faecalis* ATCC 29212) was more evident. In the samples with  $x_{Eu} = 0.1$ , the inhibition was observed for concentrations higher than 0.062 mg/ml. For the as-prepared Eu:HAp samples with  $x_{Eu} = 0.2$  the inhibition was observed for all concentrations. For *Candida albicans* ATCC 10231 a good inhibition was observed for samples with  $x_{Eu} = 0.2$  (Figure 6).

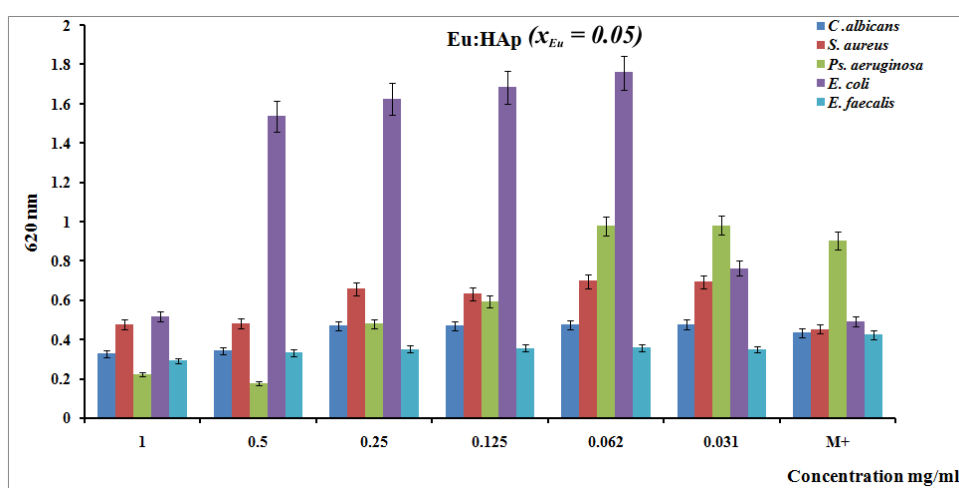


Figure 4:Antimicrobial activity of as-prepared Eu:HAp samples ( $x_{Eu} = 0.05$ ) on *E. coli* ATCC 25922, *Pseudomonas aeruginosa* 1397, *Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231.

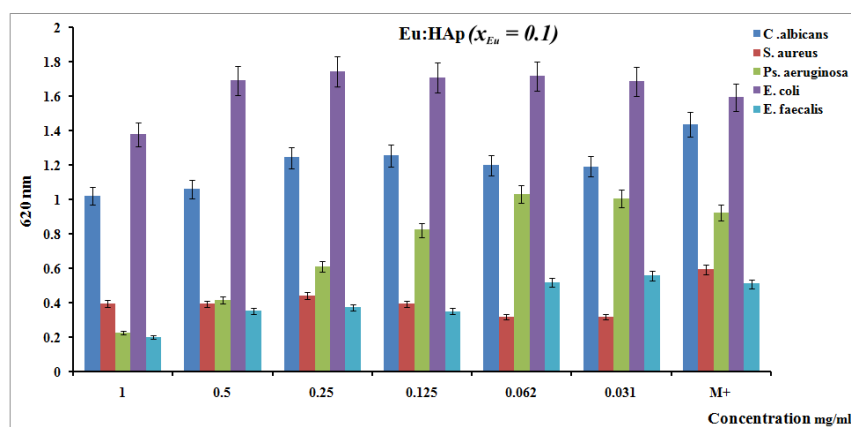


Figure 5: Antimicrobial activity of as-prepared Eu:HAp samples ( $x_{Eu} = 0.1$ ) on *E coli* ATCC 25922, *Pseudomonas aeruginosa* 1397, *Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231.

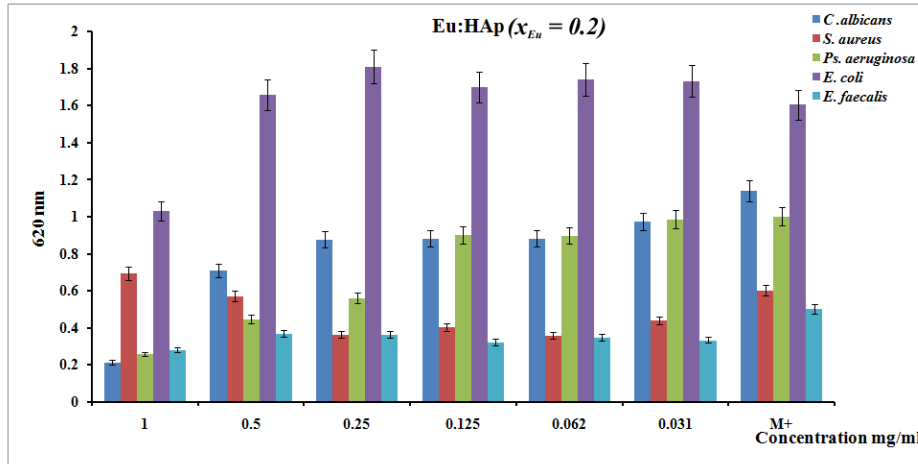


Figure 6: Antimicrobial activity of as-prepared Eu:HAp samples ( $x_{Eu} = 0.2$ ) on *E coli* ATCC 25922, *Pseudomonas aeruginosa* 1397, *Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231.

For samples with  $x_{Eu} = 0.05$  and  $x_{Eu} = 0.1$  the inhibition was observed at high concentrations (Figures 4-6). Additionally, a very good inhibition of *Pseudomonas aeruginosa* 1397 has been noticed when the concentration of Eu:HAp ( $0.05 \leq x_{Eu} \leq 0.2$ ) increased from 0.125 mg/ml to 1 mg/ml. Raimondi et al. [70] and Morones et al.[71], studying the inhibition of bacterial growth by differentially shaped nanoparticles, showed that the antimicrobial efficacy of the nanoparticles depends on the shape of the nanoparticles.

One of the aims of this study was to obtain an Eu:Hap stoichiometric apatite and to contribute to the study of the influence of europium in the structure of hydroxyapatite. We note that it is possible to determine the type of apatite, nonstoichiometric or stoichiometric, using Fourier deconvolution techniques. This study allowed highlighting the stoichiometry of Eu:HAP biomaterials, based on changes in the phosphate  $\nu_1$  and  $\nu_3$  absorbances in the 900-1200  $\text{cm}^{-1}$  spectral region. The Sauer GR et al. [72] in their studies claimed that the presence of the  $[\text{PO}_4]^{3-}$  doublet at 602 and 567  $\text{cm}^{-1}$  in all the composites suggest that the precursor phase of the HAP was octacalcium phosphate, OCP,  $(\text{Ca}_8\text{H}_2[\text{PO}_4]_6)$ . On the other hand, they

showed that the OCP precursor ensures a more crystalline and ordered HAp phase. Granja PL et al. [73] in their previous studies have affirmed that if the precursor had been amorphous calcium phosphate (ACP) ( $\text{Ca}_2[\text{PO}_4]_3$ ), these  $[\text{PO}_4]^{3-}$  bands should be a broad singlet instead of a doublet. Moreover, Hutchens SA et al. in 2006 [74] evidenced that ACP precipitation requires the rapid interaction between  $\text{Ca}^{2+}$  and  $[\text{PO}_4]^{3-}$  at high supersaturation instead of precursor complexation with other species. A previous study of apatite minerals realized by Rey et al. in 1991 [75], using Fourier deconvolution analysis, has attributed a  $1020\text{ cm}^{-1}$  band to nonstoichiometric apatite containing  $[\text{HPO}_4]^{2-}$  and  $[\text{CO}_3]^{2-}$ , and the band at  $1125\text{ cm}^{-1}$  in FT-IR deconvoluted spectra to stoichiometric apatite. Due to the presence of the band at around  $1127\text{ cm}^{-1}$  in all the prepared samples, our present studies have shown that the Eu:HAp is a stoichiometric apatite.

Moreover, our present study demonstrates that the antibacterial activity of Eu:HAp nanoparticles is dependent on the europium concentration. Furthermore, the inhibitory effect was found to be dependent to the increase of concentration from  $0.031\text{ mg/ml}$  to  $1\text{ mg/ml}$ .

## Conclusions

In the present work, we contributed to the study of the influence of europium in structure of hydroxyapatite. Using Fourier deconvolution techniques we showed that it is possible to determine the type of Eu:HAp apatite, nonstoichiometric or stoichiometric. The spectra of Eu:HAp samples in the spectral regions of  $450\text{--}700\text{ cm}^{-1}$  and  $900\text{--}1200\text{ cm}^{-1}$  were analyzed by means of second derivative and using Fourier deconvolution analysis. This study allowed highlighting the stoichiometry of Eu:HAp biomaterials, based on changes in the phosphate  $\nu_1$  and  $\nu_3$  absorbances in the  $900\text{--}1200\text{ cm}^{-1}$  spectral region.

The antimicrobial activity of Eu:HAp ( $0 \leq x_{\text{Eu}} \leq 0.2$ ) nanoparticles was tested using the most common bacterial pathogens and fungus: *E coli* ATCC 25922 (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* 0364 (Gram-positive), *Enterococcus faecalis* ATCC 29212 (Gram-positive) and *Candida albicans* ATCC 10231 (fungus).

In summary, this study on the antimicrobial activity of Eu:HAp ( $0 \leq x_{\text{Eu}} \leq 0.2$ ) nanoparticles describes a nanotechnology-based strategy luminescent  $\text{Eu}^{3+}$  doped hydroxyapatite represents a potential application for drug release and targeting based on their luminescent properties. These results and methods could be interesting for academic and

industrial researchers in biomaterials, potential orthopedic medical materials and drug carriers.

### **Competing interests**

The authors declare that they have no competing interests.

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